Granulocyte colony-stimulating factor as a potential inducer of ovulation in infertile women with luteinized unruptured follicle syndrome

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Luteinized unruptured follicle (LUF) syndrome is one of the intractable ovulation disorders that are commonly observed during cycles of treatment with ovulation inducers, for which no effective therapy other than assisted reproductive technology is available. Here, we investigated whether granulocyte colony-stimulating factor (G-CSF) could prevent the onset of LUF syndrome. We analyzed the effects of G-CSF in 68 infertile women with LUF syndrome who received ovulation induction (clomiphene + human chorionic gonadotropin (hCG) therapy or follicle-stimulating hormone + hCG therapy). G-CSF (lenograstim, 100 μg) was administered subcutaneously. Onsets of LUF syndrome were compared between the cycle during which G-CSF was given in combination with the ovulation inducer (ie, the G-CSF treatment cycle) and the subsequent cycle during which only the ovulation inducer was given (ie, the G-CSF nontreatment control cycle). The results showed that LUF syndrome recurred in only 3 cycles during the G-CSF treatment cycle (4.4% (3/68 cycles)), whereas LUF syndrome recurred in 13 cycles during the subsequent G-CSF nontreatment control cycle (19.1% (13/68 cycles)). The additional use of G-CSF significantly prevented the onset of LUF syndrome during ovulation induction ($P = 0.013$, McNemar test). No serious adverse reactions because of the administration of G-CSF were observed. In conclusion, our findings indicate that G-CSF may become a useful therapy for LUF syndrome. (Translational Research 2016;171:63–70)

Abbreviations: ART = assisted reproductive technology; FSH = follicle-stimulating hormone; G-CSF = granulocyte colony-stimulating factor; hCG = human chorionic gonadotropin; LUF = luteinized unruptured follicle; NSAIDs = nonsteroidal anti-inflammatory drugs; WBC = white blood cell

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INTRODUCTION

Ovulation disorders are one of the major causes of female infertility.[1] The selective estrogen receptor modulator (clomiphene) or gonadotropins (ie, follicle-stimulating hormone [FSH] and human chorionic gonadotropin [hCG]) are widely used for the treatment of ovulation disorders. Clomiphene is known to induce ovulation fairly well,[2] whereas adverse effects of clomiphene lead to cervical mucus insufficiency,[3] thinning of the endometrium,[4] and luteinized unruptured follicle (LUF) syndrome.[5]

AT A GLANCE COMMENTARY

Shibata T, et al.

Background

As a part of cancer chemotherapy, granulocyte colony-stimulating factor (G-CSF) has been used for at least 30 years in clinical practice for the treatment of neutropenia. No serious adverse drug reactions have been reported with the use of G-CSF, to the best of our knowledge.

Translational Significance

The present study clinically demonstrated that G-CSF is useful for infertile women diagnosed with luteinized unruptured follicle caused by ovulation induction.

LUF syndrome is an intractable ovulation disorder in which the luteinization of ovarian follicles is observed without follicle rupture or ovum extrusion.[5] Although the cause of LUF syndrome is still unknown, groups have suggested that endometriosis[6,7] or the use of nonsteroidal anti-inflammatory drugs (NSAIDs) during the periovulatory phase[8] could cause LUF syndrome. Because NSAIDs inhibit not only the production of prostaglandin but also neutrophil chemotaxis,[9,10] reduced inflammatory reactions because of NSAID use during ovulation could contribute to the onset of LUF syndrome.

A well-known hypothesis proposed by Espey[11] in 1980 focused mainly on the mechanism of ovulation as an inflammatory reaction. Neutrophils, which play a central role in inflammatory reactions, infiltrate the thecal layer during the periovulatory phase of the menstrual cycle.[12] Granulocyte colony-stimulating factor (G-CSF) is generally known as a cytokine that induces inflammatory reactions, thereby enhancing neutrophil function, and G-CSF and its receptor are known to be produced by granulosa cells.[13] We reported that the G-CSF messenger RNA levels in granulosa or theca cells were increased by at least 10-fold during the preovulatory late follicular phase compared with other phases of the menstrual cycle,[14] suggesting an important role for G-CSF in the mechanism of ovulation during the late follicular phase.

In the field of cancer chemotherapy, recombinant human G-CSF has been used for at least 30 years in clinical practice for the treatment of neutropenia. No serious adverse drug reactions have been linked to the use of G-CSF, to the best of our knowledge. Under these circumstances, we conducted the present study to evaluate whether the administration of an inducer of inflammatory reaction, G-CSF, would be feasible as a treatment for LUF syndrome.

METHODS

Patients. Infertile women who had been diagnosed with LUF syndrome at least once participated in the present clinical study. The inclusion criteria were as follows: age <40 years and no severe pelvic adhesions around the ovaries and fallopian tubes as assessed by laparoscopy. The exclusion criteria were as follows: (1) white blood cell (WBC) count ≥10,000/μL at the time of the administration of G-CSF; (2) severe disorders of the liver, kidney, or heart; (3) allergic predisposition; and (4) any other conditions assessed by the investigator as leading to the patient being ineligible for participation in the study. To evaluate the effects of the additional use of G-CSF on LUF syndrome, we compared the number of cycles showing a recurrence of LUF syndrome between the cycle during which G-CSF was given in combination with ovulation inducers (clomiphene + hCG therapy or FSH + hCG therapy; ie, the G-CSF treatment cycle) and the subsequent cycle during which only prescribed therapy was given (ie, the G-CSF nontreatment control cycle). The type, dose, and duration of each ovulation inducer were tightly equalized between the G-CSF treatment cycle and the G-CSF nontreatment control cycle.

The present study was conducted at the Department of Obstetrics and Gynecology of Kanazawa Medical University and at St. Luke Clinic from April 2006 to March 2015. Because patients with relatively uncommon LUF syndrome were enrolled in the study, the study required a longer period of time; however, the same evaluation methods were used throughout the study. Written informed consent was obtained from all study patients before the administration of G-CSF. The study was conducted after being approved by the Ethics Review Board of Kanazawa Medical University (Number 90).

Criteria for ovulation and LUF syndrome. We monitored the patients' follicular development by transvaginal ultrasonography and with measurements of their serum estradiol levels. Both ovulation and LUF syndrome were diagnosed using serial transvaginal ultrasonography in the period between the follicular and luteal phases (Fig 1). Ovulation was diagnosed if any of the following 4 criteria were met after the hCG administration: (1) reduction in the mean diameter of dominant follicles, indicating the process of follicular rupture[15]; (2) disappearance of the dominant follicles, indicating the complete rupture of follicles; (3) morphologic changes within the dominant follicles, indicating the

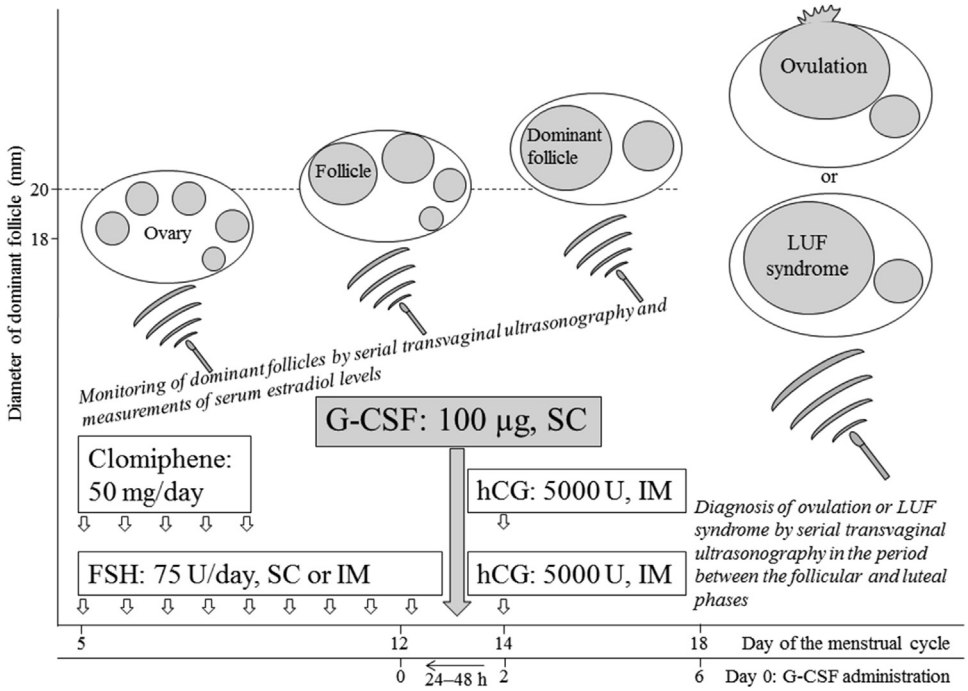

Fig 1. Diagnosis of LUF syndrome and the regimens of ovulation induction and G-CSF. Follicular development was monitored by transvaginal ultrasonography and measurements of serum estradiol levels. LUF syndrome was diagnosed using serial transvaginal ultrasonography in the period between the follicular and luteal phases. The administration of hCG was started when follicular maturation was expected to occur because the mean follicular diameter exceeded 18 mm with the serum estradiol level of >200 pg/mL. hCG was administered intramuscularly (IM) at a dose of 5000 U. G-CSF (100 μg) was administered subcutaneously (SC) within 24–48 hours before the administration of hCG. Clomiphene was administered orally once daily at a dose of 50 mg over a 5-day period starting on Day 5 of the menstrual cycle. FSH was administered SC or IM at a dose of 75 U from around Day 5 of the menstrual cycle. FSH, follicle-stimulating hormone; G-CSF, granulocyte colony-stimulating factor; hCG, human chorionic gonadotropin; LUF, luteinized unruptured follicle.

accumulation of the blood generated at the time of follicular rupture into the postovulation follicles[16]; and (4) in addition to criteria (1–3), marked echo-free space in the Douglas pouch, indicating the accumulation of follicular fluid or blood in the Douglas pouch after ovulation.[15] In some patients who had more than 1 developing follicle, ovulation was diagnosed if at least 1 follicle met any of the aforementioned criteria (1–4). LUF syndrome was diagnosed if there was no diagnostic evidence of ovulation.

Regimens. The dosing regimens for each constituent drug were as follows (Fig 1): (1) clomiphene was administered orally once daily initially at a dose of 50 mg (1 tablet) over a 5-day period starting on Day 5 of the menstrual cycle; (2) FSH was administered subcutaneously or intramuscularly initially at a dose of 75 U from around Day 5 of the menstrual cycle; (3) hCG was administered intramuscularly at a dose of 5000 U in both the clomiphene + hCG therapy and the FSH + hCG therapy groups. The administration of hCG was started when follicular maturation was expected to occur because the mean follicular diameter exceeded 18 mm with a serum estradiol level of >200 pg/mL; and (4) G-CSF (Neutrogin, lenograstim; Chugai Pharmaceutical Co, Tokyo) was administered subcutaneously at a dose of 100 μg within 24–48 hours before the administration of hCG. The timing of the G-CSF administration was defined based on the following observations: (1) an increase in the serum G-CSF level from Day 9 to 13 of the menstrual cycle during ovulation induction,[17] and, in particular, (2) an increase in the serum G-CSF level within 40 hours before the administration of hCG.[18]

Hematology. We measured the serum estradiol level (picograms per milliliter) and the serum progesterone level (nanograms per milliliter) on the day of the hCG trigger and during the midluteal phase. The WBC count (per microliter) and the serum G-CSF level (picograms per milliliter) were determined on Day 0 (the day of the G-CSF administration was defined as Day 0), 1, and 6 and thereafter. We used enzyme-linked immunosorbent assays in the measurements of the serum G-CSF levels (assay kit for human G-CSF, cat. no. 27131; Immuno-Biological Laboratories, Fujioka, Japan).

Statistical analyses. All data are presented as the mean ± standard deviation. The paired *t* tests, McNemar test, and Dunnett test (2 sided) as multiple comparisons were performed to compare paired data from the same individual patients. *P* values <0.05 were accepted as representing significant differences. IBM Statistical Package for the Social Sciences (SPSS) Statistics version 21 (IBM, Armonk, NY) was used for all statistical analyses.

RESULTS

Characteristics of patients. The overall (the clomiphene + hCG therapy group and the FSH + hCG therapy group) age of the total of 112 women with LUF syndrome was 33.3 ± 3.6 years. The overall body mass index was 21.1 ± 2.6 kg/m^2. The gravidity numbers were as follows: nulligravida, 77 of 112 patients (68.8%); once, 17 of 112 patients (15.2%); twice, 13 of 112 patients (11.6%); and thrice, 5 of 112 patients (4.5%). The parity numbers were as follows: nulliparity, 90 of 112 patients (80.4%) and once, 22 of 112 patients (19.6%). Of the 112 women, 62 (55.4%) were diagnosed with endometriosis by laparoscopy. The overall revised American Fertility Society score[19] was 10.5 ± 18.9. The overall adhesion score was 4.4 ± 8.6. Other overall results and data of the women who received the clomiphene + hCG therapy (n = 80) or the FSH + hCG therapy (n = 32) are summarized in Table I.

Effects of G-CSF to prevent LUF syndrome during ovulation induction. G-CSF was administered systemically by subcutaneous injection to 112 women with LUF syndrome in combination with an ovulation inducer (clomiphene, FSH, and hCG). Before our analysis of the effects of G-CSF, 44 women were excluded from the initial population for the following reasons: (1) 13 became pregnant during the G-CSF treatment cycle; (2) 11 switched their therapy to assisted reproductive technology during the G-CSF nontreatment control cycle; (3) 10 changed the method of ovulation induction during the G-CSF nontreatment control cycle; and (4) 10 were lost to follow-up. Of the remaining 68 women analyzed, 46 were from the clomiphene + hCG therapy group and the other 22 were from the FSH + hCG therapy group (Fig 2).

In the overall group, the incidence of LUF syndrome in the G-CSF treatment cycle (4.4% [3/68 cycles]) was significantly reduced compared with that in the G-CSF nontreatment control cycle (19.1% [13/68 cycles]; *P* = 0.013, McNemar test). From the analysis of 46 women in the clomiphene + hCG therapy group, we observed that the incidence of LUF syndrome was decreased by the additional use of G-CSF from 19.6% (9/46 cycles, the G-CSF nontreatment control cycle) to 4.3% (2/46 cycles, the G-CSF treatment cycle; *P* = 0.039, McNemar test). In the 22 women of the FSH + hCG therapy group, there were no significant differences in the incidence of LUF syndrome between the G-CSF treatment cycle (4.5%, 1/22 cycles) and the G-CSF nontreatment control cycle (18.2%, 4/22 cycles; *P* = 0.375, McNemar test; Fig 3).

In the overall group and the clomiphene + hCG therapy group, the following were not significantly different from those of the G-CSF nontreatment control cycle: (1) the day of the hCG trigger; (2) the estradiol level on the day of the hCG trigger; (3) the number of preovulatory follicles on the day of the hCG trigger; (4) the estradiol level per preovulatory follicle on the day of the hCG trigger; (5) the estradiol level during the midluteal phase; and (6) the progesterone level during the midluteal phase of the G-CSF treatment cycle. In the FSH + hCG therapy group, although the day of the hCG trigger of the G-CSF

Table I. Baseline characteristics of the 112 patients with luteinized unruptured follicle syndrome

	Overall	Clomiphene + hCG therapy group	FSH + hCG therapy group
n	112	80	32
Age (mean ± SD)	33.3 ± 3.6	33.0 ± 3.5	34.0 ± 3.7
Height (cm)	158.4 ± 5.0	158.2 ± 5.2	159.0 ± 4.5
Weight (kg)	52.9 ± 7.1	53.2 ± 7.1	52.2 ± 7.1
BMI (kg/m^2)	21.1 ± 2.6	21.2 ± 2.5	20.6 ± 2.6
Number of pregnancies (n)			
0	77 (68.8%)	55 (68.8%)	22 (68.8%)
1	17 (15.2%)	13 (16.3%)	4 (12.5%)
2	13 (11.6%)	9 (11.3%)	4 (12.5%)
3	5 (4.5%)	3 (3.8%)	2 (6.3%)
Number of deliveries (n)			
0	90 (80.4%)	63 (78.8%)	27 (84.4%)
1	22 (19.6%)	17 (21.3%)	5 (15.6%)
Laparoscopic findings (n)			
Endometriosis	62 (55.4%)	49 (61.3%)	13 (40.6%)
Fallopian tube anomaly	19 (17.0%)	13 (16.3%)	6 (18.8%)
Pelvic adhesion	8 (7.1%)	4 (5.0%)	4 (12.5%)
PCO	3 (2.7%)	2 (2.5%)	1 (3.1%)
Uterine myoma	2 (1.8%)	1 (1.3%)	1 (3.1%)
Adenomyosis uteri	1 (0.9%)	1 (1.3%)	0 (0%)
No abnormalities	2 (1.8%)	2 (2.5%)	0 (0%)
Not tested	15 (13.4%)	8 (10.0%)	7 (21.9%)
rAFS score	10.5 ± 18.9	11.9 ± 20.5	6.5 ± 12.6
Adhesion score	4.4 ± 8.6	4.4 ± 8.9	4.3 ± 7.9

Abbreviations: BMI, body mass index; *FSH,* follicle-stimulating hormone; *G-CSF,* granulocyte colony-stimulating factor; *hCG,* human chorionic gonadotropin; *PCO,* polycystic ovary; *rAFS,* revised American Fertility Society; *SD,* standard deviation.
Not tested: laparoscopy was not tested.
Percentages may not add up to 100% because of rounding.

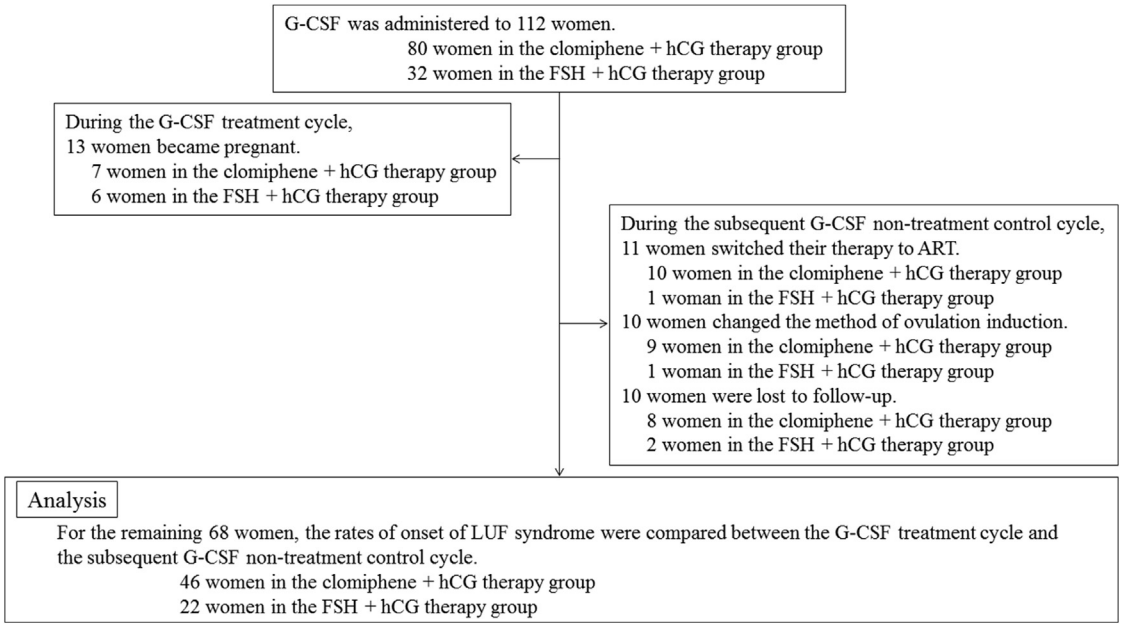

Fig 2. Overall study flow. ART, assisted reproductive technology; FSH, follicle-stimulating hormone; G-CSF, granulocyte colony-stimulating factor; hCG, human chorionic gonadotropin; LUF, luteinized unruptured follicle.

treatment cycle was slightly different from that of the G-CSF nontreatment control cycle, no significant differences in other factors were observed between the G-CSF treatment cycle and the G-CSF nontreatment control cycle (Table II).

Pregnant women. In the present study, 13 women established a clinical pregnancy during the G-CSF treatment cycle. Among the pregnant women, the treatment of 4 women was combined with embryo transfers (ETs). The clinical pregnancy rate without ET when G-CSF was used with the clomiphene + hCG therapy was 7.5% (6/80 women), and when G-CSF was used with the FSH + hCG therapy, it was 9.4% (3/32 women). The live birthrate without ET when G-CSF was used with the clomiphene + hCG therapy was 6.3% (5/80 women), and when G-CSF was used with the FSH + hCG therapy, it was 6.3% (2/32 women). The mean age of the pregnant women was 33.8 ± 3.3 years, and the laparoscopic findings of the pregnant women were as follows: (1) endometriosis (5/13 women, 38.5%); (2) fallopian tube anomaly (2/13 women, 15.4%); (3) pelvic adhesions (1/13 women, 7.7%); and (4) no history of laparoscopy (5/13 women, 38.5%). The mean revised American Fertility Society score was 11.3 ± 17.7, and the mean adhesion score was 2.8 ± 4.7. The gravidity numbers were as follows: nulligravida, 6 of 13 (46.2%); once, 4 of 13 (30.8%); and twice, 3 of 13 (23.1%). The parity numbers were as follows: nulliparity, 8 of 13 (61.5%) and once, 5 of 13 (38.5%).

Hematology and safety. The WBC count transiently increased to 19,622 ± 4991/μL on Day 1 after the start of the administration of G-CSF, but on Day 6 and thereafter, the WBC count returned to 6738 ± 1809/μL, which was comparable to the baseline WBC count, 6354 ± 1814/μL, observed on Day 0. The serum G-CSF level on Day 1 (69.6 ± 35.0 pg/mL) transiently increased compared with Day 0 (10.8 ± 4.2 pg/mL; Table III). In the present study, no serious adverse events because of the administration of G-CSF were observed.

DISCUSSION

The results from the present study of 68 patients showed the usefulness of G-CSF in preventing the onset of LUF syndrome when administered in combination with an ovulation inducer, especially in the clomiphene + hCG therapy group. The frequency of LUF syndrome was 25.1% in the patients who received clomiphene[15] and 20% in the patients who received a clomiphene + gonadotropin regimen.[20] The incidence rates of LUF syndrome in the G-CSF nontreatment control cycle in the present study (the clomiphene + hCG therapy, 19.6% and overall, 19.1%) were similar to those of the previous reports. Qublan et al[15] reported that the rate of recurrence of LUF syndrome was as high as 78.6% in patients receiving clomiphene. In the present study, the incidence rates of LUF syndrome in the G-CSF treatment cycle (the clomiphene + hCG therapy with G-CSF, 4.3%; the FSH + hCG therapy

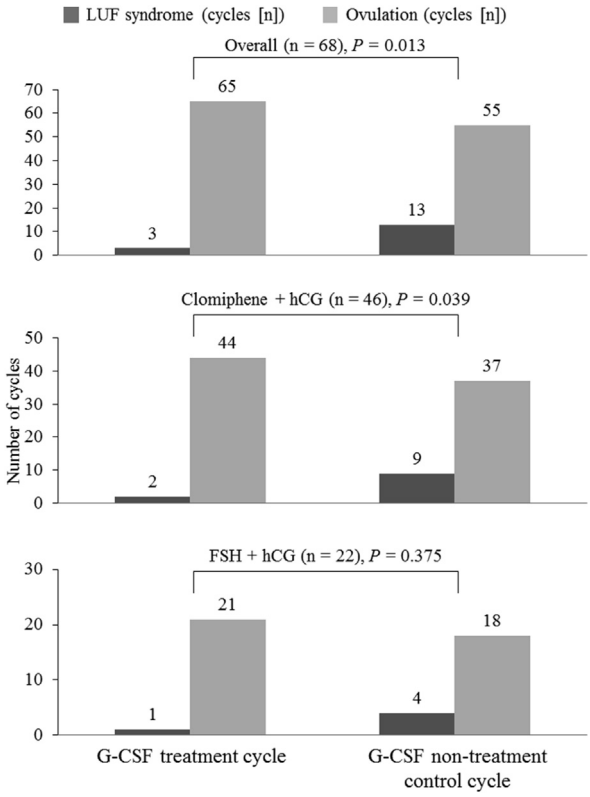

Fig 3. The ability of G-CSF to prevent LUF syndrome during ovulation induction. G-CSF significantly inhibited LUF syndrome induced by an overall ovulation inducer (the incidence of LUF syndrome: the G-CSF treatment cycle 4.4% [3/68 cycles], the G-CSF nontreatment control cycle 19.1% [13/68 cycles], $P = 0.013$). The incidence of LUF syndrome induced by the clomiphene + hCG therapy was significantly reduced by G-CSF (the G-CSF treatment cycle 4.3% [2/46 cycles], the G-CSF nontreatment control cycle 19.6% [9/46 cycles], $P = 0.039$). The additional use of G-CSF was found to reduce the rate of onset of LUF syndrome to 4.5% (1/22 cycles) during the FSH + hCG therapy. If G-CSF was not administered, however, the corresponding rate of the onset increased to 18.2% (4/22 cycles) ($P = 0.375$). All statistical analyses were performed using the McNemar test. FSH, follicle-stimulating hormone; G-CSF, granulocyte colony-stimulating factor; hCG, human chorionic gonadotropin; LUF, luteinized unruptured follicle.

with G-CSF, 4.5%; and overall, 4.4%) demonstrated that G-CSF had marked an ovulation-promoting activity.

The G-CSF level in serum transiently increased on Day 1 (69.6 ± 35.0 pg/mL), which was higher than that of the periovulatory phase in the physiological menstrual cycle (11.2 ± 1.2 pg/mL) that we reported.[21] The half-life of G-CSF after subcutaneous administration was estimated to be 6–12 hours,[22] suggesting only short-term efficacy of G-CSF in vivo. On the other hand, the half-life of neutrophils is as long as 3.75 days,[23] which can be further prolonged in the presence of G-CSF at the site of inflammation.[24] Because the present study involved the administration of hCG within 24–48 hours after the administration of G-CSF and assuming that ovulation occurred approximately 36 hours after the administration of hCG, we estimated that ovulation would occur during the period between 2.5 days (=[24 + 36 h]/24 h) and 3.5 days (=[48 + 36 h]/24 h) after the G-CSF administration. Considering the setting of the duration of the period from the G-CSF administration until ovulation in the present study, we suspect that the neutrophils with a longer half-life, rather than G-CSF with a shorter half-life, had an effect on ovulation.

The administration of leukocytes was found to markedly increase the number of ovulations in an ovarian-perfusion rat model,[25] whereas the administration of neutrophil-depleting antibodies (RP-3) reduced the ovulation rate in the same rat model.[26] These reports demonstrated that neutrophils play an important role in ovulation, particularly in the rupturing of the follicle. As the probable reason for the promotion of ovulation by G-CSF administration observed in the present study, we suggest that G-CSF may contribute to ovulation by increasing the systemic neutrophil counts, thereby increasing neutrophil counts in the thecal layer in a proportional manner.

The impact of G-CSF on pregnancy remain controversial. The clinical pregnancy rate of the clomiphene + hCG therapy with G-CSF in the present study (7.5%) showed little difference from that of the clomiphene + hCG therapy without G-CSF (8.9%), which was previously reported by Macgregor et al.[27] Scarpellini and Sbracia[28] demonstrated that the subcutaneous administration of G-CSF increased the birthrate among women suffering from habitual abortion. Gleicher et al[29,30] reported that the direct administration of G-CSF to the endometrium improved the thickness of the endometrium (<7 mm) that was unsuitable for implantation. Nonetheless, Barad et al[31] reported that intrauterine perfusions with G-CSF did not affect the clinical pregnancy rates in in vitro fertilization patients.

This study revealed the usefulness of G-CSF with neutrophil-stimulating activity as a novel therapy for LUF syndrome in clinical practice. Our findings can be expected to have an impact on current infertility treatments. This low-cost and easy-to-use G-CSF technique could be widely used in the future for the treatment of anovulatory women with repeated episodes of LUF syndrome, instead of the immediate prescription of assisted reproductive technology without careful consideration.

Table II. Characteristics of the G-CSF treatment cycle and the G-CSF nontreatment control cycle

	G-CSF treatment cycle	G-CSF nontreatment control cycle	*P* values
Overall (cycles [n] = 68)			
Day of hCG trigger			
Day of the menstrual cycle (d)	14.9 ± 3.0	15.5 ± 3.5	0.104
Estradiol (pg/mL)	568.4 ± 446.6	514.0 ± 322.9	0.290
Number of preovulatory follicles (n)	1.8 ± 1.1	1.7 ± 1.0	0.634
Estradiol per preovulatory follicle (pg/mL)	358.0 ± 173.4	433.2 ± 215.6	0.318
Midluteal phase			
Day of the menstrual cycle (d)	23.0 ± 3.5	23.4 ± 3.7	0.462
Estradiol (pg/mL)	427.7 ± 419.3	401.4 ± 366.5	0.650
Progesterone (ng/mL)	24.1 ± 19.4	25.4 ± 17.3	0.590
Clomiphene + hCG therapy group (cycles [n] = 46)			
Day of hCG trigger			
Day of the menstrual cycle (d)	14.6 ± 2.7	14.7 ± 2.3	0.831
Estradiol (pg/mL)	605.1 ± 521.2	554.1 ± 355.0	0.472
Number of preovulatory follicles (n)	2.2 ± 1.3	2.0 ± 1.2	0.622
Estradiol per preovulatory follicle (pg/mL)	344.1 ± 194.0	486.1 ± 258.7	0.263
Midluteal phase			
Day of the menstrual cycle (d)	23.0 ± 3.2	22.9 ± 2.6	0.800
Estradiol (pg/mL)	424.6 ± 345.0	470.9 ± 413.4	0.414
Progesterone (ng/mL)	29.0 ± 21.9	30.4 ± 18.4	0.666
FSH + hCG therapy group (cycles [n] = 22)			
Day of hCG trigger			
Day of the menstrual cycle (d)	15.5 ± 3.5	17.1 ± 5.0	0.027
Estradiol (pg/mL)	491.4 ± 211.9	429.9 ± 227.8	0.312
Number of preovulatory follicles (n)	1.2 ± 0.4	1.2 ± 0.4	1.000
Estradiol per preovulatory follicle (pg/mL)	377.6 ± 159.5	359.0 ± 125.1	0.727
Midluteal phase			
Day of the menstrual cycle (d)	23.0 ± 4.1	24.3 ± 5.1	0.160
Estradiol (pg/mL)	433.5 ± 543.1	268.7 ± 202.8	0.205
Progesterone (ng/mL)	14.9 ± 7.7	15.9 ± 9.5	0.728

Abbreviations: FSH, follicle-stimulating hormone; *G-CSF,* granulocyte colony-stimulating factor; *hCG,* human chorionic gonadotropin.
Values are the mean ± standard deviation. Paired *t* test was used for the statistical analyses.

Table III. Hematologic findings in the patients after the administration of G-CSF

	WBC (/μL)		G-CSF (pg/mL)	
Day*	Count	*P* value vs Day 0	Serum level	*P* value vs Day 0
0	6354 ± 1814	n.d.	10.8 ± 4.2	n.d.
1	19,622 ± 4991	<0.001	69.6 ± 35.0	<0.001
6 and thereafter	6738 ± 1809	0.611	20.9 ± 13.9	0.031

Abbreviations: G-CSF, granulocyte colony-stimulating factor; *n.d.,* not done; *WBC,* white blood cell.
Values are the mean ± standard deviation. The Dunnett test was used for the statistical analyses.
*Day 0 was defined as the day of G-CSF administration (control).

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